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Note

Gas chromatography–mass spectrometry and selected ion monitoring of the N,N'-dipentafluoropropionylhexafluoroisopropyl ester of glutamine

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Numerous methods for the determination of amino acids by gas chromatography (GC) have been developed and Hušek and Macek[1] reviewed the state of the art in 1975. In all these methods, however, glutamine is hydrolysed or solvolysed to glutamic acid or a corresponding ester. Collins and Summer[2] recently reported a GC method for the indirect determination of glutamine by measuring the amount of the pyroglutamic acid derivative which is formed during the derivatization of glutamine according to Roach and Gehrke[3]. For application to biological materials, however, this method requires prior separation of glutamine from pyroglutamic acid and is therefore not suitable for the trace analysis of glutamine. For the detection of small amounts of glutamine it is very desirable to have a method for direct derivatization and measurement.

Using the derivatization method described by Bertilsson and Costa[4] and Watson *et al.*[5], we have developed a technique for direct measurement of glutamine; chemical ionization spectra using isobutane as a reagent gas are compared with the corresponding electron impact spectra and discussed in respect to applications in selected ion monitoring.

EXPERIMENTAL

Materials

The following chemicals were of the highest purity available: glutamine (G 9003, Sigma, St. Louis, Mo., U.S.A.); glutamic acid (G 1126, Sigma); 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) (24626, Serva, Heidelberg, G.F.R.); pentafluoropropionic anhydride (PFPA) (77292, Fluka, Buchs, Switzerland); pyroglutamic acid (P 3634, Sigma).

Methods

For derivatization, glutamine or glutamic acid (100 pmoles–10 nmole) was dissolved in 100 μl of PFPA–HFIP (2:1). After 2 h at 60°, the samples were cooled to room temperature and were then ready for injection.

For gas chromatography–mass spectrometry (GC–MS) and selected ion monitoring (SIM), the following conditions were used. A Carlo Erba GI 450 gas chromatograph, Fractovap 2101 AC, was employed with a glass column (50 cm × 2 mm I.D.) packed with 3% QF-1 on Chromosorb W (80–100 mesh). The injection block temperature was 200° and the carrier gas was helium at a pressure of 0.5 kp/cm². A Vacuum Generators mass spectrometer, Micromass 16 F with Data System 2000, was used. A single jet separator was employed at 250° and the ion source temperature was 220°.

For the electron impact mode, the emission current was 50 μA and the electron energy 20 eV. For chemical ionization the reagent gas was isobutane at 10^{-5} Torr, the emission current was 100 μA and the electron energy was 50 eV.

RESULTS AND DISCUSSION

Fig. 1 shows the mass spectra and the proposed fragmentation patterns of the HFIP–PFP derivative of glutamine using (a) electron impact and (b) chemical ionization. Chemical ionization results in an MH^+ signal at *m/e* 589. The base peaks at *m/e* 230 and *m/e* 426 can be used for trace analysis. Fig. 2 shows the mass spectra and the proposed fragmentation pattern of the HFIP–PFP derivative of glutamic acid, also using (a) electron impact and (b) chemical ionization.

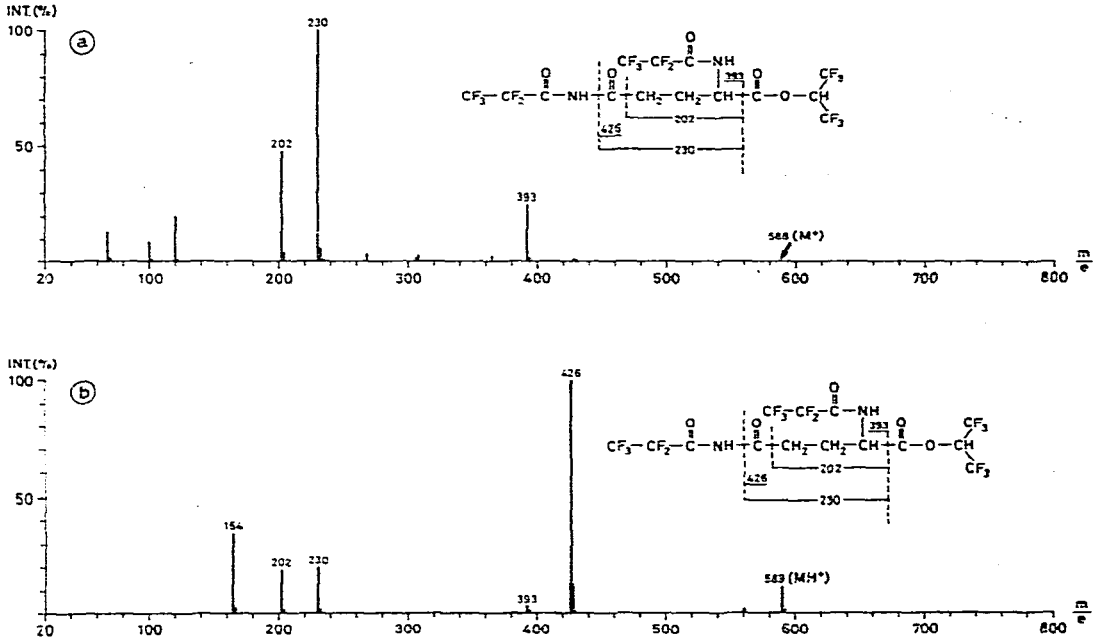


Fig. 1. (a) Electron impact and (b) chemical ionization mass spectra of the HFIP–PFP derivative of glutamine.

The fragment ion at *m/e* 426 resulting from the cleavage of the amide bond of the glutamine or the ester bond of the glutamic acid derivative occurs with both modes of ionization, but chemical ionization results in the formation of base peaks at *m/e* 426 for both compounds. The fragment ions at *m/e* 202 and *m/e* 230 can in addition be used for further confirmation in mass fragmentography, but the back-

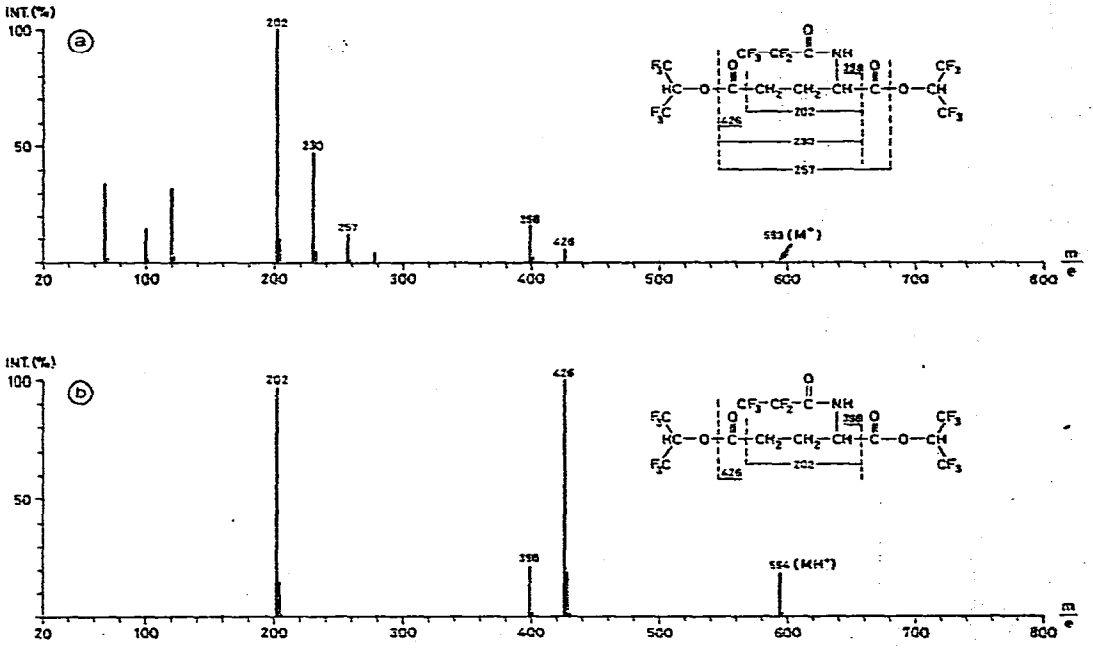


Fig. 2. (a) Electron impact and (b) chemical ionization mass spectra of the HFIP–PFP derivative of glutamic acid.

ground signals at *m/e* 202 originating from solvent impurities and from the column background were found to disturb the detection of small amounts of compounds (picogram level).

Fig. 3 shows a mass fragmentogram of the HFIP–PFP derivative of glutamic acid (1) and glutamine (4); recording was carried out at *m/e* 426 using chemical ionization. Two additional peaks (2 and 3) were found to result from derivatives of pyroglutamic acid formed from glutamic acid during the derivatization procedure.

Fig. 4 shows a mass fragmentogram of the glutamine derivative (4') monitored at *m/e* 426 using chemical ionization; no formation of glutamic acid or pyroglutamic acid derivatives could be observed. This result is contrary to that reported by Collins and Summer[2], who used the derivatization procedure in acidic *n*-butanol at 100°, which led to the conversion of glutamine into pyroglutamic acid *n*-butyl ester.

Fig. 5 shows a mass fragmentogram of the glutamine derivative when monitoring was carried out at *m/e* 230 using electron impact; the detection limit was 0.3 pmoles. If chemical ionization was used, the detection limit on monitoring at *m/e* 426 (base signal) was 3 pmole.

Chemical ionization gave lower background signals for all *m/e* values that were tested for selected ion monitoring. However, this finding did not help in achieving a better detection limit.

The derivatives were found to be stable for at least 1 week if stored at room temperature and in the absence of moisture.

For the investigation of glutamine in tissues or body fluids, enough material is usually available to carry out determinations using ion-exchange chromatography methods originating from the work of Stein and Moore[6]. In addition, high-perform-

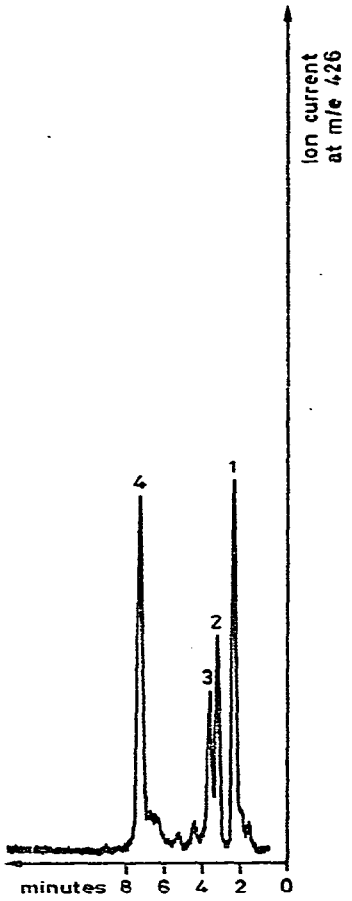
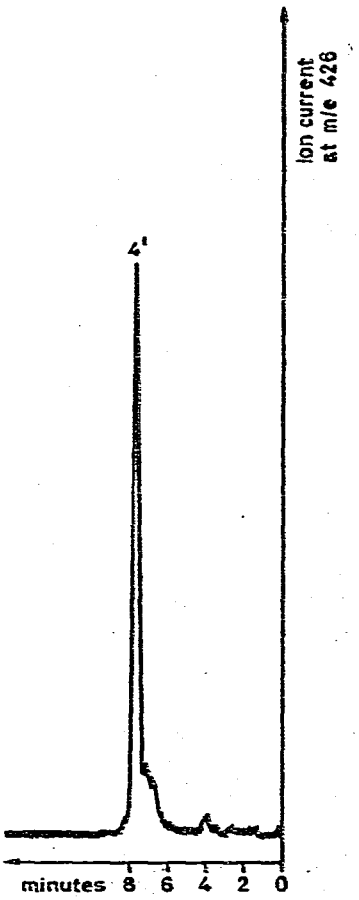


Fig. 3. Mass fragmentogram from HFIP–PFP derivatives of glutamic acid (*ca.* 100 pmoles peak 1) and glutamine (100 pmoles, peak 4) monitored at *m/e* 426 using chemical ionization. Gas chromatography was carried out with temperature programming from 120° to 170° at 4°/min.

ance liquid chromatography is a powerful technique for investigations of amino acids, including glutamine[7]. None of these methods, however, can match the detection limit and specificity of selected ion monitoring.

For the investigation of the metabolism of glutamine and glutamic acid in brain samples, a very sensitive and specific method is required. As pyroglutamic acid is a major degradation product of glutathione[8], the latter compound is present in millimolar concentrations in brain samples[9], and the method reported by Collins and Summer[2] is not suitable for the measurement of trace amounts of glutamine in brain samples.

By the use of different stable isotope precursors, the methods presented here can be used for the investigation of the metabolism and compartmentation of glutamine and glutamic acid in brain samples as reported by Van den Berg[10], and the well known problems of specificity in liquid scintillation spectrometry could be eliminated.



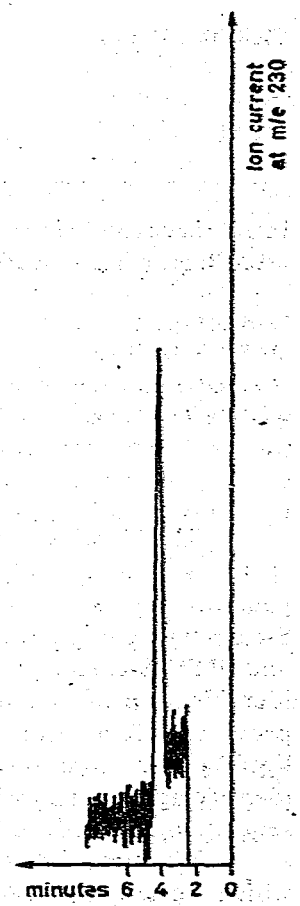


Fig. 4. Mass fragmentogram from the HFIP–PFP derivative of glutamine (200 pmoles, peak 4') monitored at *m/e* 426 using chemical ionization. Gas chromatography was carried out with temperature programming from 120° to 170° at 4°/min.

Fig. 5. Mass fragmentogram from the HFIP–PFP derivative of glutamine (2 pmoles) monitored at *m/e* 230 using the electron impact mode. Gas chromatography was carried out isothermally at 150°.

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